UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Leukaemia Diagnosis Interpretation
All Participants

Participant: 42981 **Trial No: 151602**

Date Issued: 07-September-2015 **Closing Date: 25-September-2015**

Trial Comments

Your Diagnosis: Chronic lymphocytic leukaemia/small lymphocytic lymphoma

Consensus Diagnosis: Chronic lymphocytic leukaemia/small lymphocytic lymphoma

Your Diagnosis is classed as a **CORRECT** result

Performance Score: 0

Running Score: 50

Consensus

	Consensus Diagnosis	Your Diagnosis
Lineage	Lymphoid-B cell	Lymphoid-B cell
Subclassification	Mature (peripheral) B-cell neoplasms	Mature (peripheral) B-cell neoplasms
Diagnosis	Chronic lymphocytic leukaemia/small lymphocytic lymphoma	Chronic lymphocytic leukaemia/small lymphocytic lymphoma

Breakdown of returned diagnoses

Diagnosis	No Of Returns	Percentage Of Returns
Total Returns	291	100.0
Chronic lymphocytic leukaemia/small lymphocytic lymphoma	285	97.9
B-cell prolymphocytic leukaemia	4	1.4
Diffuse large B-cell lymphoma(DLBCL), NOS	1	0.3
Primary DLBCL of the CNS	1	0.3

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Leukaemia Diagnostic Interpretation trial 151602

Case History

A 68 year old male was referred by his GP following a consultation for 'flu-like' symptoms which had not improved over a number of weeks. The patient also had a loss of appetite and had experienced weight loss. A subsequent examination found lymphadenopathy and splenomegaly.

Haematological examination revealed the following:

Hb- 127 g/L; WBC- 66.07 x10^9/L; PLT- 265 x10^9/L; RBC- 4.04 x10^12/L; HCT- 0.405 L/L; MCV- 100.2 fL; MCH- 31.4 pg

Immunophenotype

The consensus results for the malignant cells had the following phenotype (consensus percentage expression in brackets – corrected to the nearest whole percent):

Positive Antigens - CD5 (87%), CD11c (35%), CD19 (97%), CD20 (44%), CD23 (54%), CD43 (95%), CD45 (100%), CD200 (93%), HLADR (91%), Kappa (95 %)

Negative Antigens - CD2 (1%), CD3 (1%), CD10 (0%), CD13 (0 %), CD22 (13%), CD25 (0%), CD38 (9%), CD79b, (30%), FMC7 (19%)

Cytogenetics and Molecular genetics

FISH analysis of 100 interphase nuclei showed deletion of TP53 at 17p13.1

Histology

A lymph node core biopsy showed that the normal architecture had been replaced by a diffuse lymphoid infiltrate. This was composed of small cells with occasional scattered large cells. Proliferation centres were not well developed.

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Peripheral Blood Morphology

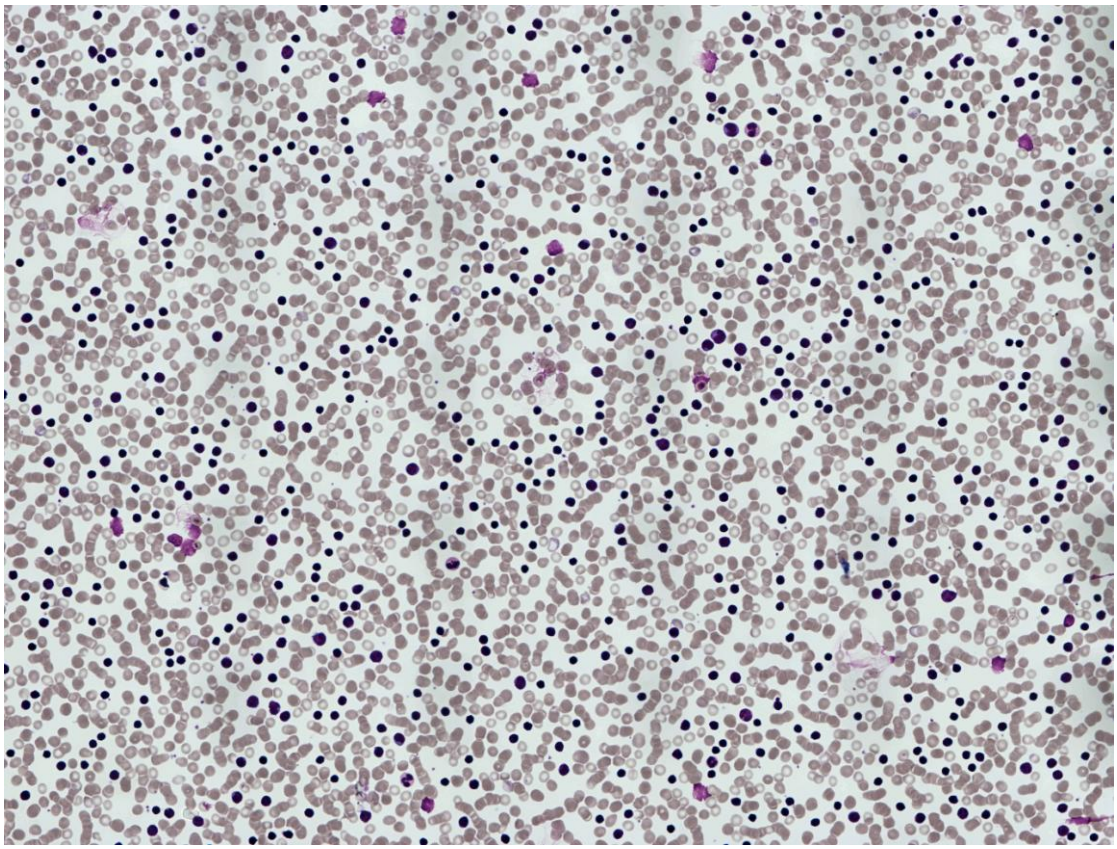
Morphology comments from Professor Wendy Erber
Consultant Haematologist, University of Western Australia, Nedlands, Australia

COMMENT:
There is a peripheral blood lymphocytosis. The majority (approximately 80%) are small round cells with a high nuclear:cytoplasmic ratio and coarse chromatin. The remaining 20% of cells are larger with a lower nuclear:cytoplasmic ratio, less nuclear chromatin condensation and a single prominent nucleolus. Smears cells are present as well as there being a left shift.

Diagnosis: Chronic Lymphocytic Leukaemia (CLL) with 20% prolymphocytes.

Examples of Digital Images Issued

Figure 1: Peripheral Blood x50 magnification (May Grünwald Giesma stain)



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EXERCISE CONCLUSION/CASE DISCUSSION

Please note that, because this trial requires a diagnosis based on the WHO guidelines, this report is therefore based on the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th Edition[1].

LEUKAEMIA IMMUNOPHENOTYPING - Part one

UK NEQAS LI was contacted by two participants following the issue of the LI 151602 report querying the fact that CD79b had a negative consensus but the percentage positivity was 30.08%. The results shown on the report are both qualitative and quantitative. The consensus positive/negative is qualitative and is derived from the ratio of positive to negative results entered by participants with the report showing the consensus result. The robust mean (30.08%) is quantitative and is derived from the percentage positive results entered.

Because the qualitative result is modal, CD79b was classed as negative because 51% of participants entered a negative result. Analysis of the results showed that 17 participants who entered a positive result had also entered <30% positivity (2 participants who entered positive had <10% positivity), whereas 5 participants entered a negative result for >30%.

LEUKAEMIA DIAGNOSTIC INTERPRETATION - Part Two

Exercise Consensus Diagnosis (Classified as Correct)

Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL)

Other Differential Diagnoses (Classified as Major Errors):

B-cell prolymphocytic leukaemia (B-PLL)
Diffuse large B-cell lymphoma (DLBCL), NOS
Primary DLBCL of the CNS

Other Differential Diagnoses (Classified as Minor Errors):

None for this trial

The classification of major and minor errors is currently under review and will be discussed with our steering committee in the near future.

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Rationale for Correct Classification

Morphologically and phenotypically, this was a standard case of Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) with a *TP53* deletion at 17p13.1 which is indicative of a poor prognosis.

Please note that for this case to be B-PLL, the majority of cells should be prolymphocytes (>55%, usually >90%). With reference to the morphological comments from Professor Wendy Erber, this case has 20% prolymphocytes. There are significant differences in the phenotype between CLL/SLL and B-PLL. There is strong expression of CD19, CD20, CD22, CD79b and FMC7 in B-PLL whereas this case had week expression of CD20, CD22 and CD79b and was negative for FMC7, typical of CLL/SLL. In cases of B-PLL, CD5 positivity is seen in 20% to 30% with CD23 being expressed in 10% to 20%. At least half of B-PLL cases would have the *TP53* mutation but complex karyotypes are also common. Cases of B-PLL (especially with the *TP53* mutation) have a different clinical course and therapy compared to CLL/SLL.

This case cannot be classed as DLBCL, NOS based on the morphology and the phenotype. Only 10% of cases express CD5 and morphologically the cells are not large B-cells. The *TP53* mutation is not found in this disease. This rationale can also be applied to the diagnosis of Primary DLBCL of the CNS. Additionally, there were no clinical signs, or reference to CNS infiltration (intracerebral/intraocular) in our accompanying narrative.

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Further Trial Findings

Table 1: Submissions from all participants - laboratories and individuals.

Diagnosis	Total No. of Returns	No. of Laboratories	No. of Individual
Chronic lymphocytic leukaemia/small lymphocytic lymphoma	285	195	90
B-cell prolymphocytic leukaemia	4	1	3
Diffuse large B-cell lymphoma(DLBCL), NOS	1	0	1
Primary DLBCL of the CNS	1	0	1

Table 2: Submissions from laboratory participants only

Diagnosis	Laboratory Returns %
Chronic lymphocytic leukaemia/small lymphocytic lymphoma	99.5
B-cell prolymphocytic leukaemia	0.5

Table 3: Submissions from individual participants only

Diagnosis	Individual Returns %
Chronic lymphocytic leukaemia/small lymphocytic lymphoma	94.7
B-cell prolymphocytic leukaemia	3.2
Diffuse large B-cell lymphoma(DLBCL), NOS	1
Primary DLBCL of the CNS	1

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References:

1. Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th Edition. IARC Press 2008

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Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600, Fax: +44 (0) 114 267 3601
e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance. Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided.

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